American Fern Journal 100(1):45–53 (2010)

Isoëtes laosiensis, a New Species from Lao PDR

CHANGKYUN KIM
Department of Biological Science, Ajou University, Suwon 443–749, Republic of Korea
SOMCHANH BOUNPHANMY
Department of Biology, Faculty of Science, National University of Laos, Dongdok, P.O. Box 7322, Vientiane, Lao PDR
BYUNG–YUN SUN
Division of Biological Science, Chonbuk National University, Jeonju 561–756, Republic of Korea
HONG–KEUN CHOI*
Department of Biological Science, Ajou University, Suwon 443–749, Republic of Korea

ABSTRACT.—An undescribed species of *Isoëtes* (Isoëtaceae) was found during a floristic survey in Lao PDR. This Laotian species is distinctive enough to identify it as a separate species from the related taxon *I. coromandelina* by its megaspore characteristics, polyhedral shape, large tubercules on proximal and distal surfaces, and the uniform size. We propose *I. laosiensis* as a new species from South East Asia (Laos).

KEY WORDS.—*Isoëtes*, *Isoëtes laosiensis*, Lao PDR, new species

During the last decade botanical research has become active in Laos (Newman *et al.*, 2007a, b; Thomas *et al.*, 2007). Recently the largest output of floristic research is "A Checklist of the Vascular Plants of Lao PDR" (Newman *et al.*, 2007b), which incorporates data from historical specimen collections in European herbaria as well as more recent botanical field work. However, the distribution of *Isoëtes* species has not yet been reported in this area (Pfeiffer, 1922; Newman *et al.*, 2007a, b). During a recent field survey in Laos, we found an unrecorded *Isoëtes* species similar to *I. coromandelina* L. (*s.l.*) in general appearance.

Isoëtes coromandelina was first described from the Coromandel Coast, Tamil Nadu, India in 1781 by C. Linneaeus, fil. This species has been well documented, with detailed investigations of its mega and microspore morphologies, which have been among the main characters used in delimiting this and other Indian species of *Isoëtes* (Pant and Srivastava, 1962; Singh *et al.*, 1983; Srivastava *et al.*, 1993). Marsden (1976) reported a new subspecies, *I. coromandelina* ssp. *macrotuberculata* Marsden based on the ornamentation of the megaspores (i.e., larger tubercles on the proximal face and irregularly corrugated ridges).

Our comparison between Laotian *Isoëtes* plants and *Isoëtes* species from India and North Australia revealed differences in the ornamentation of spores

* Corresponding author; fax: +82 31 219 1795, e-mail: hkchoi@ajou.ac.kr

(Pfeiffer, 1922, Pant and Srivastava, 1962; Marsden, 1976; Srivastava *et al.*, 1993; Cook, 1996), and the former are here described as a new species.

Materials and Methods

We collected materials of an unrecorded *Isoëtes* species from Nagang village, Laos (N 14° 46′ 8.8″, E 106° 1′ 44.9″). Voucher specimens are deposited at AJOU. For delimitation of this unrecorded species, we compared it with the original descriptions and herbarium specimens of *I. coromandelina (s.l.)*, *I. indica* Pant & Srivastava, *I. sampathkumaranii* Rao, *I. dixitei* Shende, *I. mahadevensis* Srivastava, Pant, & Shukla, *I. panchganiensis* Srivastava, Pant, & Shukla, and *I. panchananii* Pant & Srivastava from India and North Australia (Table 1 and Appendix 1). As diagnostic morphological characters we examined the number of corm lobes, the number of leaves, leaf length and width, the number of peripheral fiber strands, presence or absence of stomata and velum, shape of labium and ligule, and sporangium length and width (Table 1). Observations, measurements, and photomicrographs of mega and microspores were performed by an Olympus BX40 microscope and a Jeol JSM–6380 scanning electron microscope.

Results and Discussion

Morphology.—The morphological characters for our newly describes species, *I. laosiensis*, and *Isoëtes* species from India and North Australia are listed in Table 1. *Isoëtes laosiensis* differs from Indian *Isoëtes* species, *I. coromandelina*, *I. sampathkumaranii*, *I. mahadevensis*, *I. panchganiensis*, and *I. panchananii*, in the number of corm lobes, presence or absence of peripheral fiber strands in the sporophyll, presence or absence of velum, and shape of labium and ligule. The number of corm lobes has been used in the taxonomy of *Isoëtes* (Pfeiffer, 1922; Takamiya *et al.*, 1997). *Isoëtes laosiensis* has a three lobed corm, whereas *I. mahadevensis* and *I. panchananii* typically have two lobed corms (Pant and Srivastava, 1962; Srivastava *et al.*, 1993). The peripheral fiber strand is a mechanical tissue which consists of a few short fibers and situated under the epidermis in the sporophyll (Takamiya *et al.*, 1997). *Isoëtes laosiensis* has them at four sites, whereas no such strand is found in *I. sampathkumaranii*, *I. mahadevensis*, and *I. panchananii* (Pant and Srivastava, 1962; Srivastava *et al.*, 1993). A velum is absent in *I. laosiensis* but those of *I. sampathkumaranii*, *I. panchganiensis*, and *I. panchananii* are more or less well developed covering about half to all of the surface of the sporangium (Pant and Srivastava, 1962; Srivastava *et al.*, 1993). The labium of *I. laosiensis* is absent or very short, extending to 1/5 of the total ligule length, whereas the labium of *I. coromandelina (s.l.)* is conspicuous, hemiorbicular, covering all but the apex of the ligule (Table 1). The ligules of *I. laosiensis* and *I. coromandelina* were similar, being elongated deltoid in shape (Fig. 1C), whereas those of *I. indica*, *I. dixitei*, *I. mahadevensis*, *I. panchganiensis* and *I. panchananii* have been described as triangular or deltoid with a cordate base (Table 1).

The measurements of morphological characters from our new species, including the number of leaves, leaf length and width, presence or absence of stomata, and sporangium length and width are consistent with those previously observed in *Isoëtes* species from India and North Australia (Pfeiffer, 1922, Pant and Srivastava, 1962; Marsden, 1976; Cook, 1996). Therefore, it is difficult to distinguish species by many morphological characters. In many cases, vegetative characters of *Isoëtes* are considered of limited taxonomic value because of their infraspecific variability or their interspecific uniformity (Hickey, 1986a). It has been also well documented that many vegetative characters vary as the result of phenotypic plasticity (Johnson, 1984; Cook, 1996; Britton *et al.*, 1999). This could be why we did not determine the key characters for delimitation of *I. laosiensis* based on its external appearance.

Spore ornamentation and size.—Identification of *Isoëtes* species largely rests on megaspore and microspore ornamentation and spore size (Pfeiffer, 1922; Hickey, 1986b; Macluf *et al.*, 2006; Choi *et al.*, 2008). Megaspores of *I. laosiensis* have a tuberculate ornamentation on both the proximal and distal surfaces (Fig. 2A–D). Based on the figures in Pant and Srivastava (1962) and Srivastava *et al.* (1993), there are four quillworts in India with tuberculate megaspores: *I. coromandelina*, *I. indica*, *I. sampathkumaranii*, and *I. dixitei* (Table 1). The tubercules in the megaspore of *I. laosiensis* are mostly rounded, while those in the megaspores of *I. indica* are gradually tapering towards their pointed ends (Pant and Srivastava, 1962). Moreover, the commissural ridges in megaspores of *I. laosiensis* are straight and smooth, while they are sinuous in *I. indica* (Table 1). The surface of megaspores in *I. sampathkumaranii* and *I. dixitei* shows a number of uneven tubercles both on the proximal and the distal sides, but a few or a single tubercle is seen in each pyramic area on the proximal side of *I. laosiensis* (Table 1 and Fig. 2B). Megaspore ornamentation of *I. laosiensis* is similar to that of *I. coromandelina*. However, megaspore ornamentation of *I. laosiensis* differs from those of *I. coromandelina* ssp. *coromandelina* in having markedly larger tubercles on both the proximal and distal surfaces (Fig. 2A–H). Although it is similar to *I. coromandelina* ssp. *macrotuberculata*, it differs because of its triradiate and commissural ridges, with *I. laosiensis* being straight and *I. coromandelina* ssp. *macrotuberculata*, irregularly corrugate (Table 1). Moreover, megaspores of *I. laosiensis* are uniform in size (595.0 ± 36.9 μm), whereas megaspores of *I. coromandelina (s.l.)* are always distinctly dimorphic in a single sporangium (Pant and Srivastava, 1962; Marsden, 1976) (Table 1 and Fig. 2A, E).

Microspores ornamentation of *I. laosiensis* is a laevigate on proximal surface and echinate on the distal faces (Fig. 2I–L). In texture, the microspores of *I. laosiensis* are similar to *I. coromandelina* (*s.l.*). However, *I. laosiensis* microspores (34.6 ± 2.2 μm) are larger than *I. coromandelina* (26–33 μm), although there is some overlap in the size ranges (Table 1).

Our spore morphological data indicate that *I. laosiensis* is distinctive for identification as separated species from *I. coromandelina* (*s.l.*) and the other *Isoëtes* species in India and North Australia (Table 1). The following is description of previously unnamed species of *Isoëtes*:

TABLE 1. Comparison the morphological characters of *I. laosiensis* with the previous descriptions of *Isoëtes* in India and North Australia.

Morphological characters	*laosiensis*	*coromandelina* ssp. *coromandelina*[a, b]	*coromandelina* ssp. *macrotuberculata*[b]	*indica*[a]
No. of corm lobes	3	3 (rarely 4, 5)	3 (rarely 4, 5)	3 (rarely 4)
No. of leaves	21 – 42	20 – 60	15 – 60	9 – 35
Leaf length (cm)	20.6 ± 3.8	< 60	60 – 80	8 – 55
Leaf width (mm)	2.1 ± 0.5	ND	ND	ND
Peripheral fiber strand	4	4	4	4 – 6
Stomata	present	present	present	present
Velum	absent	absent	absent	absent
Labium	absent or very short	conspicuous, hemiorbicular	conspicuous, hemiorbicular	ND
Ligule shape	elongated deltoid	elongated deltoid	lanceolate	deltoid with cordate base
Sporangium length (mm)	5.4 ± 1.2	< 12	< 12	6 – 19
Sporangium width (mm)	3.5 ± 0.4	< 9	< 7	4 – 9
Megaspore ornamentation	tuberculate (globular large tubercles)	tuberculate (numerous globular small tubercles)	tuberculate (large and small globular tubercles)	tuberculate (tubercles tapering mostly with polar end)
Megaspore diameter (um)	595.0 ± 36.9	small (356 – 458) large (465 – 660)	small (330 – 410) large (420 – 530)	small (89 – 380) medium (407 – 509) large (458 – 636)
Megaspore ridges	straight and smooth	straight and smooth	irregularly corrugate	sinuous
Microspore ornamentation	smooth with spines	smooth or rugulose to papillate or spines	ND	finely tuberculate
Microspore length (um)	34.6 ± 2.2	26–33	ND	16 – 48

Isoëtes laosiensis C. Kim & H.–K. Choi, ***sp. nov.***—TYPE: Laos, Ban Kiat Nagang village, Mt. Phou Asha (N 14° 46′ 8.8″, E 106° 1′ 44.9″), elev. 150–300 m, 24 July 2007, B.Y. Sun and S.S. Choi 2039–5 (Holotype, AJOU; Isotype, AJOU). **Figs. 1 and 2**.

Megasporae triangularis in proximalis aspectus, monomorphicae, 533.9–(595.0)–697.8 μm diam., hemisphaerium proximalis et distalis major tuberculares, juga triradiata et commissuralia laevis.

Plant amphibious. Corm globose, 3–lobed with abundant dichotomous roots. Sporophylls white basally, green above, ascending, 9.7–(20.6)–27.0 cm long,

TABLE 1. Extended.

dixitei[a]	*sampathkumaranii*[a]	*mahadevensis*[c]	*panchganiensis*[c]	*panchananii*[a]
3	ND	2 (rarely 3)	3	2
ND	ND	5 – 29	9 – 20	4 – 38
ND	ND	< 14	7 – 13	7 – 24
ND	ND	1.0 – 1.5	1.2 – 3.0	ND
present	Absent	absent	ND	absent
ND	ND	present	present	present
rudimentary (rarely covering about half of the sporangium)	present (covering about half to 2/3 of sporangium)	rudimentary	present (covering nearly the entire sporangium)	present (covering about half of sporangium)
ND	ND	triangular with obtuse apex	triangular	ND
deltoid with cordate base	ND	triangular with lobbed base	triangular with expended base	triangular
ND	ND	3 – 4.5	3 – 4	3 – 5
ND	ND	2 –2.5	2.5 – 3	2 – 3
tuberculate (uneven tubercles)	tuberculate–cristate	rugulose or pustule	reticulate	reticulate
small (320 – 458) large (483 – 660)	small (280 – 381) large (356 – 458)	small (203 –372) large (384 – 507)	small (185 – 305) large (270 – 456)	small (240 – 330) large (330 – 407)
straight	straight or slightly sinuous	undulating	straight or sinuous	straight or slightly sinuous
ND	ND	echinate	ND	ND
ND	ND	10 – 47	ND	ND

ND, not determined by original authors.
[a]Pant and Srivastava (1962);
[b]Marsden (1976);
[c]Srivastava *et al.* (1993).

1.0–(2.1)–3.0 mm wide at mid–length, in tufits of 21 to 40, half–terete, base alate. Peripheral fiber strands present. Stomata anomocytic, 67.2–(73.4)–79.1 μm long, 25.2–(35.7)–51.1 μm wide, 6–8 peristomatic neighbouring cells. Velum absent. Labium absent or very short, deltoid segment, broad at base. Ligule membranaceous, elongated deltoid. Sporangia orbicular to ovovate, wall pale to brownish, 3.3–(5.4)–8.2 mm long, 2.6–(3.5)–4.3 mm wide.

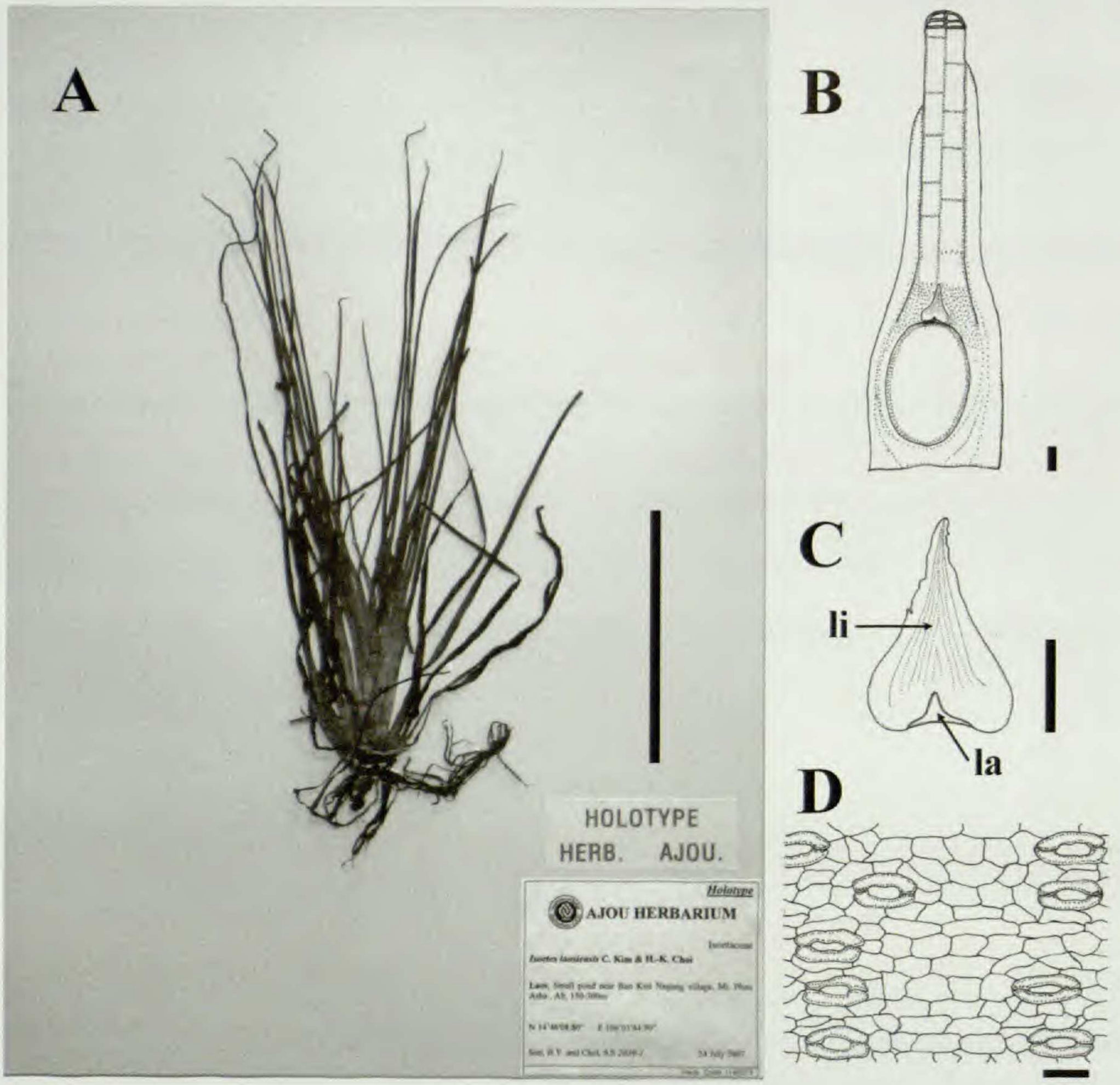

FIG. 1. *Isoëtes laosiensis* C. Kim & H.–K. Choi, *sp. nov.* **A.** Habit of plant (Nagang villiage, Laos, 24 July 2007, Sun and Choi *2039–1* [Holotype]). **B.** Adaxial view of basal portion of sporophyll. **C.** Shape of labium and ligule. la, labium; li, ligule. **D.** Stomata. Scale bars: A = 10 cm; B and C = 1 mm; D = 50 μm.

Megaspores triangular in proximal view, large tubercules on both the proximal and distal surfaces, white in dry, grey in wet, 533.9–(595.0)–697.8 μm diameter, 399.5–(480.0)–671.2 μm height, triradiate and commissural ridges straight and smooth, ultrastructure fibrous. Microspore brown, 29.2–(34.6)–40.4 μm long, 20.5–(26.0)–34.3 μm diameter, proximal hemisphere laevigate, distal hemisphere echinate, ultrastructure rugulae and granules.

ETYMOLOGY.—This species epithet *laosiensis* is derived from the name for Laos, on which the new species has been collected.

DISTRIBUTION.—This species is known only from Laos, but will be probably be found to occur widely than now recorded. At present, *Isoëtes* is poorly known through South East Asia, possibly in part because of difficulty in field recognition of the genus.

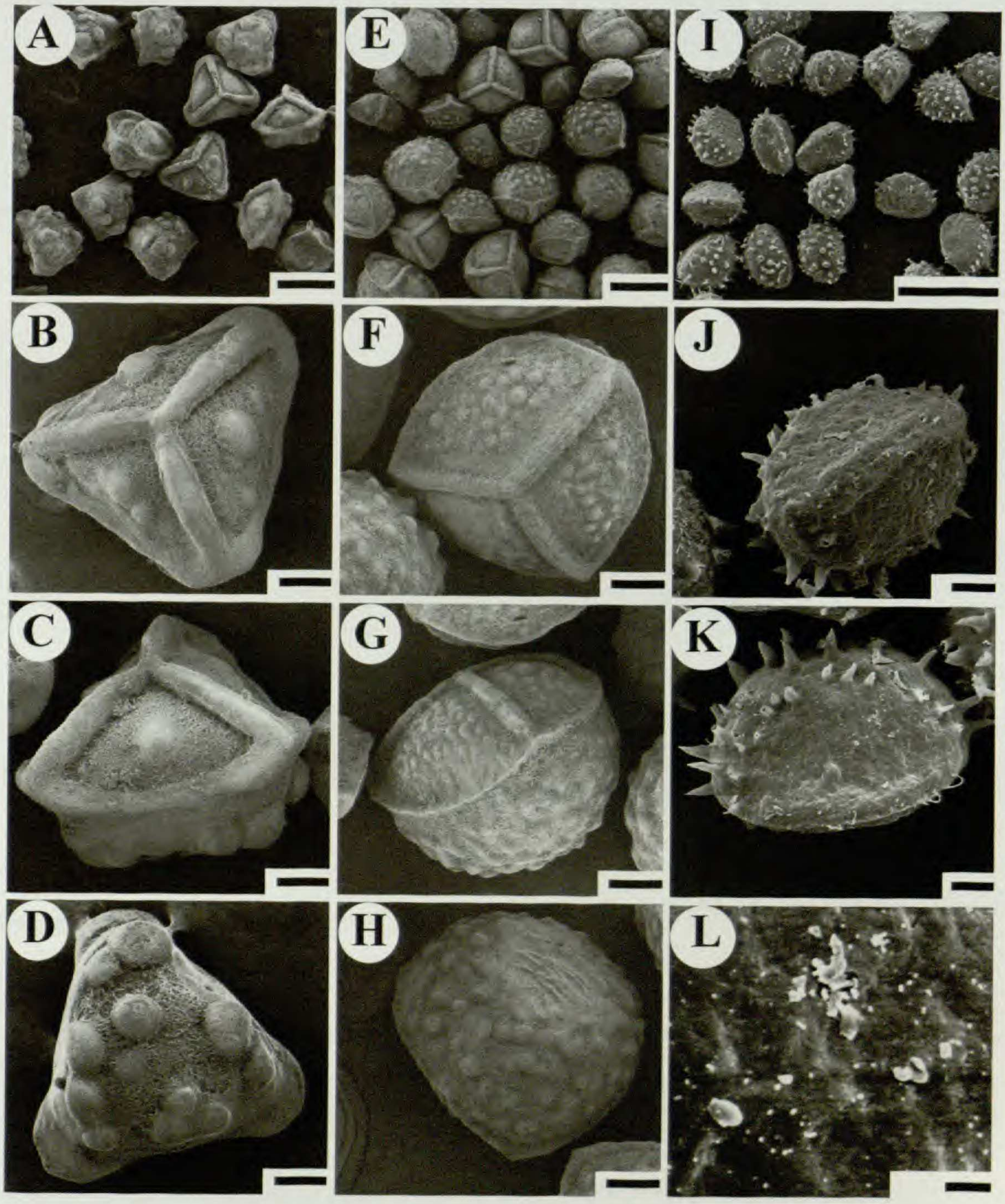

FIG. 2. Spore morphology of *I. laosiensis* C. Kim & H.–K. Choi, sp. nov. and *I. coromandelina* L. fil. **A–D.** Megaspores of *I. laosiensis* (Sun and Choi *2039–5* [Isotype]). **A.** Overview. **B.** Proximal view. **C.** Lateral view. **D.** Distal view. **E–H.** Megaspores of *I. coromandelina* (India. Rajasthan, Sept 29 1995, C.D.K. Cook *5327* [K]). **E.** Overview. **F.** Proximal view. **G.** Lateral view. **H.** Distal view. **I–L.** Microspores of *I. laosiensis* (Sun and Choi *2039–5* [Isotype]). **I.** Overview. **J.** Proximal view. **K.** Lateral view. **L.** Ultrastructure of microspore surface. Scale bars: A and E = 500 μm; B–D and F–H = 100 μm; I = 50 μm; J–K = 5 μm; L = 1 μm.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the curators and directors of the herbaria K and E, for providing access to herbarium specimens. We also thank to two anonymous reviewers for comments on the manuscript. This work was supported in part by grants from the Ministry of Environment as parts of the Eco–Technopia 21 project (KIEST 052-071-049) to HKC and by International Cooperation Unit of Biodiversity and Environmental Conservation (ICUBEC) to BYS.

LITERATURE CITED

BRITTON, D. M., D. F. BRUNTON and S. S. TALBOT. 1999. *Isoëtes* in Alaska and the Aleutians. Amer. Fern J. 89:133–141.

CHOI, H–K., J. JUNG and C. KIM. 2008. Two new species of *Isoëtes* (Isoëtaceae) from Jeju Island, South Korea. J. Plant Biol. 51:354–358.

COOK, C. D. K. 1996. *Aquatic and wetland plants of India*. Oxford University Press.

HICKEY, R. J. 1986a. On the identity of *Isoëtes triquetra* A. Braun. Taxon 35:243–246.

HICKEY, R. J. 1986b. *Isoëtes* megaspore surface morphology: Nomenclature, variation, and systematic importance. Amer. Fern J. 76:1–16.

JOHNSON, E. R. L. 1984. Taxonomic revision of *Isoëtes* L. in Western Australia. J. Roy. Soc. West. Aust. 66:28–43.

LINNAEUS, C. and FILIUS. 1781. Supplementum Plantarum, 447. Systema Vegetabilium, vol. 8. Brunsvigae.

MACLUF, C. C., M. A. MORBELLI and G. E. GIUDICE. 2006. Microspore morphology of *Isoëtes* species (Lycophyta) from Southern South America. The Botanical Review 72:121–134.

MARSDEN, C. R. 1976. A new subspecies of *Isoëtes coromandelina* from Northern Australia. Contrib. Herb. Austr. 24:1–10.

NEWMAN, M., P. THOMAS, S. LANORSAVANH, S. KETPHANH, B. SVENGSUKSA and V. LAMXAY. 2007a. New records of angiosperms and pteridophytes in the flora of Laos. Edinburgh Journal of Botany 64:225–251.

NEWMAN, M., S. KETPHANH, B. SVENGSUKA, P. THOMAS, V. LAMXAY and K. ARMSTRONG. 2007b. A Checklist of the Vascular Plants of Lao PDR. Royal Botanic Garden Edinburgh.

PANT, D. D. and G. K. SRIVASTAVA. 1962. The genus *Isoëtes* in India, Proc. Natl. Inst. Sci. India, Pt. B. Biol. Sci. 28:242–280.

PFEIFFER, N. E. 1922. Monograph of the Isoëtaceae. Ann. Missouri Bot. Gard. 9:79–232.

SINGH, R., D. R. BOHRA and B. D. SHARMA. 1983. Mixed sporangia in *Isoëtes coromandelina* L. Pollen et Spores 25:41–47.

SRIVASTAVA, G. K., D. D. PANT and P. K. SHUKLA. 1993. The genus *Isoëtes* L. in India. Amer. Fern J. 83:105–119.

TAKAMIYA, M., M. WATANABE and K. ONO. 1997. Biosystematic studies on the genus *Isoëtes* (Isoëtaceae) in Japan. IV. Morphology and anatomy of sporophytes, phytogeography, and taxonomy. Acta Phytotax. Geobot. 48:89–122.

THOMAS, P., K. SENGDALA, V. LAMXAY and E. KHOU. 2007. New records of conifers in Cambodia and Laos. Edinburgh Journal of Botany 64:37–44.

APPENDIX 1. Herbarium specimens examined in this study. Herbaria abbreviations: Royal Botanic Garden, Kew (K) and Royal Botanic Garden, Edinburgh (E).

***Isoëtes coromandelina* L. fil. ssp. *coromandelina*—India.** Kunigal, Sept 16 1969, D.A. Govindappa *120* (K); Mysore, Oct 27 1970, F.M. Jarrett *592* (K); same locality, Nov 26 1971, S.S. Hooper & C. Saldanha *s.n.* (K); Kerala, Dec 16 1985, V.S. Manickam & K.M. Matthew *s.n.* (K); same locality, Dec 21 1985, V.S.

Manickam and K.M. Matthew *s.n.* (K); Rajasthan, Sept 29 1995, C.D.K. Cook *5327* (K); Meerut, unknown date, S.N. Bhambie *1955* (K). **Sri Lanka.** Uva Province, Dec 26 1976, R.B. & A.J. Fadan *76/603* (K); Northern Provinve, Jan 13 1977, R.B. & A.J. Fadan *77/160*, *77/236* (K); Ceylon, Dec 20 1881, C. Trimen *7/82* (K).

***I. coromandelina* ssp. *macrotuberculata* Marsden—Australia.** Northern Territory, May 22 1983, P.A. Fryxell & L.A. Craven *4249* (E).

***I. dixitei* Shende—India.** Mysore, Oct 15 1969, K.B. Sadanaudra & S.N. Ramaswamy *204* (K); same locality, Aug 18 1973, S.S. Patil *2* (K).

***I. indica* Pant & Srivastava—India.** Madhya Pradesh, Nov 13 1960, D.D. Pant *2G* (K).

***I. panchananii* Pant & Srivastava—India.** Madhya Pradesh, unknown date, D.D. Pant *1* (K); same locality, Nov 13 1960, D.D. Pant *1A*, *1G* (K).

***I. sampathkumaranii* Rao—India.** Chikkamagalur, May 12 1968, S.N. Ramasaramy *118* (K).